Phytosterol Enrichment of Rice Bran Oil by a Supercritical Carbon Dioxide Fractionation Technique

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ABSTRACT: Supercritical carbon dioxide ($SC\text{-}CO_2$) fractionation technique was evaluated as an alternative process for reducing the free-fatty-acid (FFA) content and minimizing the phytosterol loss of rice bran oil (RBO) during the process. The effects of pressure (20.5 to 32.0 MPa) and temperature (45 to 80 °C) for isothermal operation of the column on the composition of the resultant fractions were examined. Low-pressure and high-temperature conditions were found to be favorable for minimizing triglycerides (TG) and phytosterol losses during the FFA removal from crude RBO. Rice bran oil fractions with < 1% FFA, about 95% TG, and 0.35% free sterol with 1.8% oryzanol content could be obtained utilizing the described $SC\text{-}CO_2$ fractionation technique.

Key Words: deacidification, phytosterol, rice bran oil, $SC\text{-}CO_2$ fractionation

Introduction

PHYTOSTEROLS ARE MINOR COMPONENTS OF ALL VEGETABLE oils comprising major portions of the unsaponifiable fraction of the oil. The cholesterol-lowering effect of sterols, which were isolated from soybeans, was first shown by Peterson (1951) in the 1950s. Later numerous studies reported the hypocholesteremic potency of plant sterols (phytosterols) (Subbiah 1973; Hassan and Rampone 1979; Gylling and others 1995; de Deckere and Korver 1996; Miettinen and Gylling 1997; Jones and Ntanios 1998; Hallikainen and Uusitupa 1999). Although phytosterol-enriched margarines have been used in Finland in recent years, they have only lately become important as a functional food ingredient in the United States due to the increased consumer demand for such nutraceuticals/functional foods.

Rice bran oil (RBO) is an excellent source of nutritionally beneficial compounds, such as sterols, tocopherols, and tocotrienols (Kahlon and others 1992; Sugano and Tsuji 1997; Nicolosi and others 1997; Westrate and Meijer 1998; Moldenhauer and others 1999). Sterols occur in plants both in the free and esterified form, the latter esterified to other moieties, such as fatty acids, glucosides, and ferulic acid. Ferulic acid esters of phytosterols are commonly known as oryzanol. Rice bran oil is rich in oryzanol and β-sitosterol (Itoh and others 1973). Oryzanol has been reported to have diverse health effects, including a hypolipidemic effect, growth promotion, and stimulation of the hypothalamus (Seetharamiah and Chandrasekhara 1989; Rukmini and Raghurum 1991; Nicolosi and others 1992.).

The biologically active components of RBO are concentrated in the unsaponifiable fraction of the oil. According to Orthoefer and Nicolosi (1993), the RBO unsaponifiable fraction contains about 43% phytosterols, 10% steryl esters, and 1% tocopherol. Ferulic acid ester content of rice bran oil unsaponifiable fraction is about 20% (Nicolosi and others 1992). The FFA content of crude RBO is higher than that of many other vegetable oils because of the presence of high levels of active lipase, which promotes hydrolysis of oil to FFA. Refining of RBO results in oil losses of 18% to 22% (w/w of total oil) during the conventional oil processing (Orthoefer and Nicolosi 1993). Furthermore, conventional refining processes to remove FFA can result in a significant reduction (about 50%) of the above-noted active RBO components (Orthoefer 1996).

High-pressure extraction and fractionation technology employing supercritical carbon dioxide ($SC\text{-}CO_2$) is an alternative technique for oil extraction and refining. Several studies have reported $SC\text{-}CO_2$ extraction of RBO. Taniguchi and others (1987) noted the presence of oryzanol in $SC\text{-}CO_2$-extracted RBO, which had a lighter color and less phosphorous than hexane-extracted oil. Zhao and others (1987) showed that fractions obtained at high extraction pressures contained low FFA, waxes, and unsponifiables. Ramsay and others (1991), comparing yields and sterol content of the hexane- and $SC\text{-}CO_2$-extracted RBO, showed that total sterol content of the $SC\text{-}CO_2$-extracted RBO was less than that found in the hexane-extracted oil. Shen and others (1996), investigating the pilot scale $SC\text{-}CO_2$ extraction of RBO, determined the apparent partition coefficients of oil components between the oil and CO_2 phases. The same research group also reported a 2-stage $SC\text{-}CO_2$ process that involved extraction of RBO in the 1st stage, followed by continuously feeding the initial extract to a 2nd-stage expansion column to achieve further fractionation of the oil components (Shen and others 1997). The rate of RBO extraction with $SC\text{-}CO_2$ has been correlated with dimensionless Sherwood, Schmidt, and Reynolds numbers by Kim and others (1999).

Fractionation of fatty acids from other vegetable-oil components (deacidification) can also be accomplished with $SC\text{-}CO_2$. Toward this end, lampante olive oil refining (Bondioli and others 1992) and deacidification of roasted peanut (Ziegler and Liaw 1993) and olive oil (Brunetti and others 1989) with $SC\text{-}CO_2$ have been reported. However, deacidication of crude RBO using a supercritical fluid fractionation (SFF) tower approach has not been reported up to date. Therefore, the objective of this study was to deacidify commercially extracted crude RBO using a packed fractionation tower and to determine the optimal conditions for FFA removal while minimizing phytosterol and triglyceride (TG) losses during the process.

Materials and Methods

CRUDE RBO, WHICH WAS USED AS FEED MATERIAL FOR THIS study, was obtained from Riceland Foods Inc. (Stuttgart, Ark., U.S.A.). This crude oil was centrifuged at 3000 rpm for 20 min, and the resultant precipitate separated from the oil prior to the SFF experiments.

Triglycerides, FFA, and steryl-fatty-acid-ester (StE) contents of the samples were analyzed by HPLC according to Moreau and others (1996). The HPLC system consisted of a pump (Model SP 8800, Spectra-Physics, San Jose, Calif., U.S.A.), an evaporative light-scattering detector (ELSD) (Alltech 500, Alltech Associates, Deerfield, Ill., U.S.A.), an autosampler (Model SpectraSYSTEM AS 3000, ThermoQuest Inc., San Jose, Calif., U.S.A.), and a column heater (BioRad Inc., Richmond, Calif., U.S.A.). ChromQuest software (Version 2.5.1, ThermoQuest Inc.) was utilized for data acquisition and system control.

Lipid and sterol components in the oil samples were separated on a LiChrosorb Diol, 5 µm, 100 × 3-mm column (Chrompack Inc., Raritan, N.J., U.S.A.). The mobile phase gradient, consisting of hexane/acetic acid/2-propanol, is described in Table 1a. Eluent flow rate was constant at 0.5 mL/min. The ELSD detector was operated at 40 °C with nitrogen as a nebulizing gas at a flow rate of 1.60 L (STP)/min. The column heater temperature was set at 40 °C.

Oil samples were dissolved in hexane (about 20 mg/mL), and a 10 µL injection volume was used. Hexane, 2-propanol and acetic acid were HPLC-grade solvents and purchased from Fisher Scientific (Fairlawn, N.J., U.S.A.). Standards of oryzanol (CTC Organics, Atlanta, Ga., U.S.A.), stigmastanol (TCI, Tokyo Kasei), cholesteryl stearate (Nu-Check-Prep Inc., Elysian, Minn., U.S.A.), β-sitosterol and campesterol (Sigma, St. Louis, Mo., U.S.A.), α- and δ-tocopherol (Aldrich Chemical Co., Milwaukee, Wis., U.S.A.), and γ- and β-tocopherol (Matreya, Inc., Pleasant Gap, Pa., U.S.A.) were used in this study. A mixture of TG, diglyceride (DG), and a FFA mixture consisting of C16:0, C18:0, C18:2 (Sigma) were used for peak identification.

Free sterol and oryzanol compositions of the samples were determined by supercritical fluid chromatography (SFC). A SFC chromatograph (Lee Scientific Model 600, Dionex Corp., Salt Lake City, Utah, U.S.A.) equipped with a SB-Phenyl-50 capillary column (10 m × 100 µm i.d., 0.5 µm film thickness, Dionex Corp.) and an integrator (Data Jet-CH2, Spectra-Physics) were also used in this study. The carrier gas was SFC-grade carbon dioxide (Air Products, Inc., Allentown, Pa., U.S.A.). All oil components were detected and quantified by a flame-ionization detector (FID) held at 350 °C. The oven temperature was kept at 100 °C. The injector valve sample loop (Valco Inc., Houston, Texas, U.S.A.) volume and injection time were 200 nL and 1 s, respectively. The pressure program used for the analysis is described in Table 1b. Triglyceride and FFA composition of the samples were reported as HPLC area percentages, whereas oryzanol, StE, and free sterols were expressed as weight percentages unless otherwise stated. Each sample was injected at least twice, and the average of the 2 analyses reported.

Table 1a—HPLC solvent gradient

Time (min)	A	B
0	100	0
5	100	0
12	75	25
40	75	25
41	100	0
60	100	0

Solvent A: hexane/acetic acid, 1000/1 (v/v)
Solvent B: hexane/2-propanol, 100/1 (v/v)

Table 1b—SFC pressure program

Initial pressure (MPa)	Ramp (MPa/min)	Hold (min)	Final pressure (MPa/min)
10	—	5	10
10	0.5	—	15
15	0.2	—	18
18	0.5	—	28
28	−10	—	10

Column fractionation

The SFF experiments were carried out on a pilot-scale column shown schematically in Fig. 1. The column has a preheater and 4 separately heated zones, each having a height of 41.5 cm and 1.43 cm i.d. The total height and volume of the column were 164 cm and 260 cm^3, respectively. The column was packed with protruded stainless-steel material (0.16 in Pro-Pak, Scientific Development Company, State College, Pa., U.S.A.), which provides 94% void volume in the column. A detailed description of the column design, and temperature and pressure control systems is given by King and others (1997).

Carbon dioxide (welding-grade, National Welding Supply, Bloomington, Ill., U.S.A.) was introduced into the column before the feed (30 mL crude RBO) was pumped into the system. The column was allowed to equilibrate at the desired temperature before pressurization. The extract collection valve was opened after desired temperature and pressure was equilibrated in the column. The column was then operated for 3 h in the semi-continuous mode, that is substrate feed was in a batch mode, while CO_2 was added in continuous mode. The duration of the fractionation experiments was 3 h unless otherwise stated. The CO_2 flow rate was 1.2 L/min as measured at room temperature and atmospheric pressure. Extract and raffinate samples were collected from the top and bottom of the column, respectively. The column was depressurized, and residual oil was drained at the end of each run. Then the column was cleaned at 34.0 MPa and 90 °C with flowing CO_2 for more than 6 h.

Statistical analysis

All fractionation runs and analysis of each extract and raffinate sample were carried out in duplicate and in randomized order, and means were reported. Analysis of variance (ANOVA) of the main effects of temperature and pressure as well as their interactions on the TG, FFA, free sterols, oryzanol, and StE content of extract and raffinate samples was performed using General Linear Model procedure of Statistix- software (Version 4.1, Analytical Software, Tallahassee, Fla., U.S.A.). Multiple comparison of the means were carried out by LSD (Least Significant Difference) test at p = 0.05.

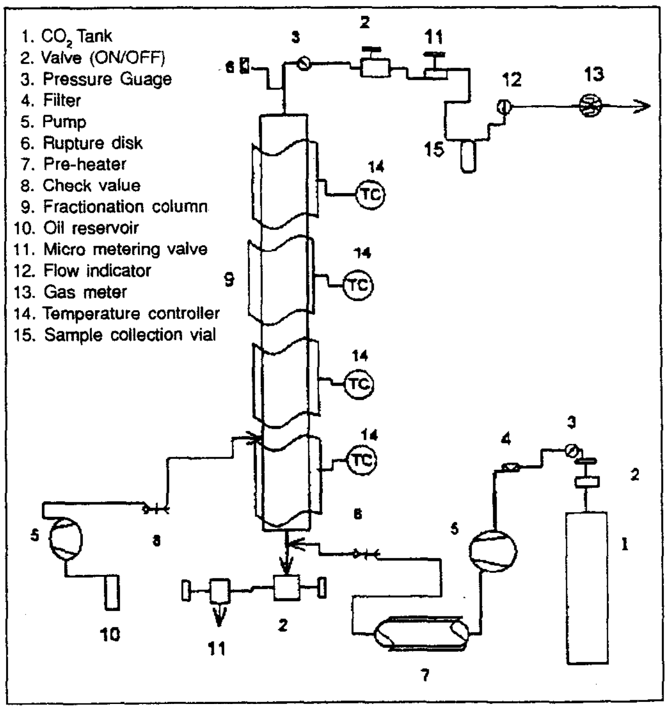

Fig. 1—Fractionation tower flow chart

Results and Discussion

TRIGLYCERIDE, FFA, AND PHYTOSTEROL CONTENTS OF THE FEED oil (Table 2) were similar to the crude RBO compositions reported by Orthoefer and Nicolosi (1993). The amount of extract collected increased with pressure and decreased with temperature (Fig. 2). The largest amount of extract (2.0 g) was collected at the highest CO_2 density studied (0.923 g/mL at 32.0 MPa and 45 °C), probably due to the higher lipid solubility in SC-CO_2 at higher CO_2 density.

Triglycerides

Triglyceride content of the extracts decreased significantly with temperature at the lower pressures (20.5 and 27.5 MPa) (Fig. 3a). For the lowest CO_2 density studied (0.6 g/mL at 20.5 MPa and 80 °C), the TGs were at the lowest concentration (about 37%) in the extract. This was due to the higher solubility as well as selectivity for FFA in SC-CO_2 under these conditions (Fig. 3b). Similar results were reported for the SC-CO_2 deacidification studies performed with the other vegetable oils (Brunetti and others 1989; Ziegler and Liaw 1993). Changes in TG content of the extracts with temperature were not significant ($p > 0.05$) at 32.0 MPa (Fig. 3a).

Although the TG content of the extracts changed significantly with temperature at 20.5 MPa (Fig. 3a), these changes were not reflected in the TG content of the raffinate (Fig. 4a). This was due to the small amount of extract (0.2 to 0.5 g) removed from the feed material (27.5 g) under these conditions (Fig. 2). The TG concentration of raffinate samples (> 72 %, Fig. 4a) was significantly higher than that of the extract samples (< 60%, Fig. 3a) and was slightly higher than that of the feed material (about 70%, Table 2) at all pressures and temperatures.

Free fatty acids

Free-fatty-acid content of the extracts, which were collected at 32.0 MPa, were lower than those obtained at the lower pressures (Fig. 3b), probably due to the higher SC-CO_2 selectivity for TG at higher pressures. The extract with the highest FFA concentration (36.6%) was obtained at 20.5 MPa and 80 °C. Free-fatty-acid content of the raffinate samples decreased with increasing pressure (Fig. 4b) because of the higher amount of extract collected at higher pressures (Fig. 2). The FFA content of the extracts was significantly higher than that of the raffinate samples. The main effect of temperature and pressure and the temperature-pressure interaction effect on the FFA content of the samples were significant ($p < 0.05$).

Free sterols

Table 3 shows the free-sterol content of extract and raffinate fractions. Free-sterol content of the extract samples was significantly higher than that of the raffinate samples at all pressure and temperature conditions studied. However, sterol concentration of raffinate fractions (0.23% to 0.35%) was still similar to that

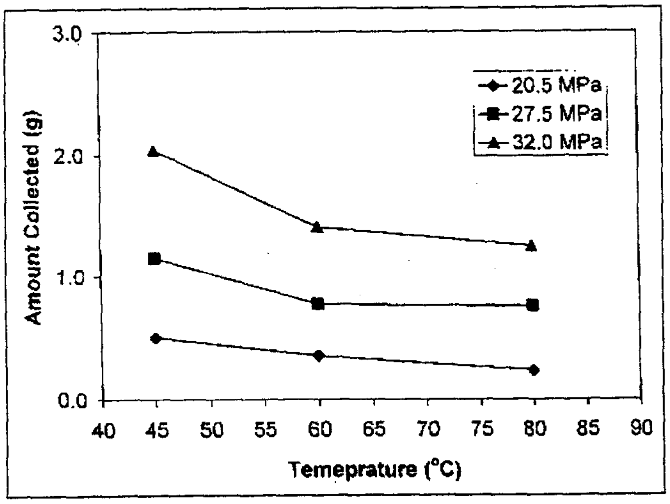

Fig. 2—Effect of temperature and pressure on the yield of extract collected during the SC-CO_2 fractionation of rice bran oil. The processing time was 3 h.

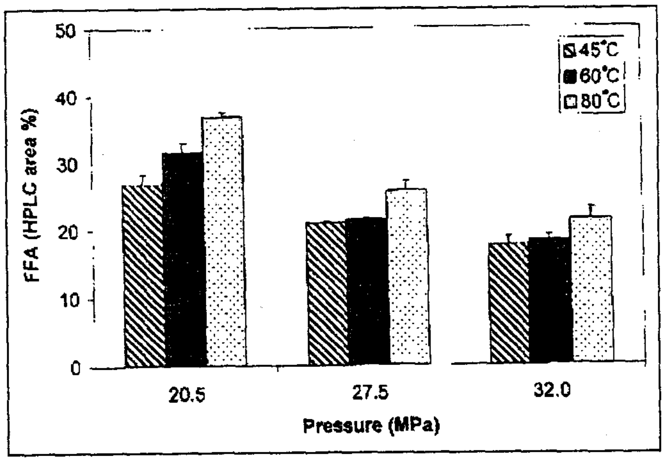

Fig. 3b—Effect of temperature and pressure on the FFA (HPLC area %) content of the extract fractions. The processing time was 3 h.

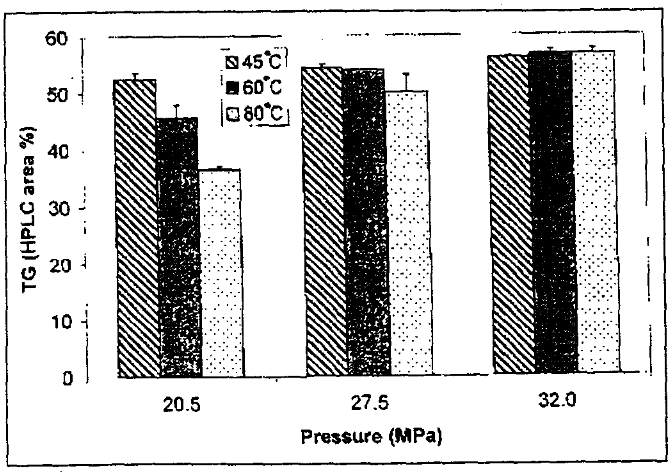

Fig. 3a—Effect of temperature and pressure on the TG content (HPLC area %) of the extracts. The processing time was 3 h.

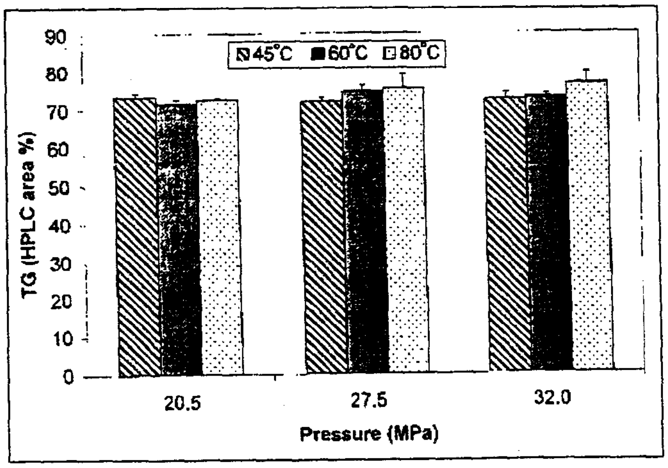

Fig. 4a—Effect of temperature and pressure on the TG (HPLC area %) content of the raffinate fractions. The processing time was 3 h.

Table 2—Crude rice bran oil composition

Triglycerides (HPLC area %)	70 ± 2
Free Fatty Acids (HPLC area %)	7.0 ± 0.5
Oryzanol (wt %)	1.3 ± 0.1
Free sterols (wt %)	0.33 ± 0.03
Steryl fatty acid esters (wt %)	3.6 ± 0.3

Table 3—Effect of pressure and temperature on the free-sterol content of the extract and raffinate fractions

Pressure (MPa)		Temperature (°C)		
		45	60	80
Extract[1,2]	20.5	0.76^{g,f}	0.83^{g}	0.70^{e,f}
	27.5	0.58^{d}	0.64^{d,e}	0.76^{g,f}
	32.0	0.47^{c}	0.59^{d}	0.69^{e,f}
Raffinate[1,2]	20.5	0.28^{a,b}	0.35^{b}	0.29^{a,b}
	27.5	0.29^{a,b}	0.29^{a,b}	0.27^{a,b}
	32.0	0.24^{a}	0.23^{a}	0.26^{a}

[1]Free-sterol content of the fractions was given as percent of the total fraction weight.
[2]Means with the same letter are not significantly different ($p > 0.05$).

Table 4—Effect of temperature and pressure on the amount of phytosterol removed with the extract fraction

Pressure (MPa)	20.5		32.0	
Temperature (°C)	45	80	45	80
Total extract amount collected (g)	0.50	0.23	2.04	1.25
Extract free sterol content (%, w/w)	0.76	0.7	0.47	0.69
Amount of free sterol removed with the extract (mg)	3.80	1.61	9.59	8.63
Extract oryzanol content (%, w/w)	0.24	0.45	0.47	0.38
Amount of oryzanol removed with the extract (mg)	1.20	1.03	9.59	4.75
Extract StE content (%, w/w)	1.65	2.79	3.27	1.78
Amount of StE removed with the extract (mg)	8.25	6.40	66.1	22.3

of the feed material (0.33%) (Table 2). The amount of free sterol removed with the extract fraction increased with increasing pressure and decreasing temperature (Table 4). The temperature-pressure interaction effect on the free-sterol content of the fractions was significant ($p < 0.05$).

Oryzanol

The oryzanol content of the raffinate samples was significantly higher (3 to 5 fold) than that of the extract fractions (Table 5). The implication of this finding is quite important for the application of SFF technology to rice bran oil deacidification because rice bran oil, which is refined using conventional processes, does not contain a significant amount of oryzanol (Table 6). Although the oryzanol content of both extract and raffinate fractions did not show a regular trend with temperature and pressure, the amount

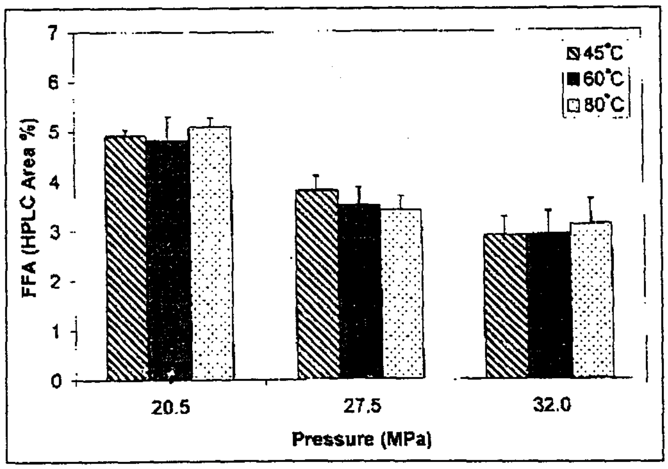

Fig. 4b—Effect of temperature and pressure on the FFA (HPLC area %) content of the raffinate fractions. The processing time was 3 h.

Table 5—Effect of temperature and pressure on the oryzanol content of the fractions

Pressure (MPa)		Temperature (°C)		
		45	60	80
Extract[1,2]	20.5	0.24^{a}	0.24^{a}	0.45^{c}
	27.5	0.26^{a,b}	0.27^{a,b}	0.22^{a}
	32.0	0.47^{c}	0.39^{b,c}	0.38^{b,c}
Raffinate[1,2]	20.5	1.35^{d,e,f}	1.41^{e,f,g}	1.29^{d,e}
	27.5	1.31^{d,e}	1.37^{d,e,f}	1.26^{d}
	32.0	1.58^{h}	1.54^{g,h}	1.47^{f,g,h}

[1]Oryzanol content of the fractions was given as percent of the total fraction weight.
[2]Means with the same letter are not significantly different ($p > 0.05$).

Table 6—Lipid and phytosterol composition[1] of rice bran oil samples deacidified using different processes[2]

Sample	TG	FFA	Free Sterol	Oryzanol	StE
Regular rice bran oil[3]	96.1^{a}	0.07^{a}	0.20^{a}	n.d.[4]	2.9^{a}
High oryzanol rice bran oil[5]	95.3^{a}	0.05^{a}	0.22^{a}	0.60^{a}	3.0^{a}
SFF processed rice bran oil[6]	95.2^{a}	0.08^{a}	0.35^{b}	1.78^{b}	2.3^{b}

[1]SFC area percentage
[2]Means in the same column with the same letter are not significantly different ($p > 0.05$).
[3]Commercially refined using conventional caustic refining
[4]n.d. = not detected
[5]Commercially refined using special techniques that were not revealed by the processor
[6]The raffinate fraction from a SFF experiment, which was carried out at 13.6 MPa, 45 °C, and 1.2 L/min CO_2 flow rate

Table 7—Effect of temperature and pressure on the sterol-fatty-acid-ester content of SC-CO_2 fractions

Pressure (MPa)		Temperature (°C)		
		45	60	80
Extract[1,2]	20.5	1.65^{a}	2.28^{b}	2.79^{b,c}
	27.5	2.76^{b,c}	2.72^{b}	2.81^{b,c}
	32.0	3.27^{d,e}	2.78^{b,c}	1.77^{a}
Raffinate[1,2]	20.5	3.55^{e,f}	3.84^{f,g,h}	4.00^{f,g,h}
	27.5	3.64^{f,g,h}	3.25^{c,d,e}	4.06^{g,h}
	32.0	3.52^{e,f}	3.96^{f,g,h}	4.18^{h}

[1]Steryl-fatty-acid-ester content of the fractions was given as percent of the total fraction weight.
[2]Means with the same letter are not significantly different ($p > 0.05$).

of oryzanol removed with the extract fraction was higher at low temperature and high pressures (Table 4).

Sterol fatty acid esters

Sterol-fatty-acid-ester contents of the raffinate samples were significantly higher than that of the extract samples (Table 7). Extracts collected at 20.5 MPa and 45 °C and 32.0 MPa and 80 °C had lower StE contents than the other pressure and temperature combinations studied, probably due to the higher SC-CO_2 selectivity towards the other oil components, that is, TG and FFA. The amount of StE removed with the extract was higher at higher pressures (Table 4). A similar trend was observed with the oryzanol and free-sterols contents of the extract fractions. Increasing sterol solubility with increasing pressure in SC-CO_2 has been reported previously for pure sterol systems by other researchers (Stahl and Glatz 1984; Wong and Johnston 1986). Wong and Johnston (1986) also reported increasing sitosterol solubility with increasing temperature. In our study, the opposite trend was observed with temperature, although the effect of temperature on the amount of phytosterol extraction was less pronounced that that of the pressure (Table 4). The difference between our results and literature might be due to a dependence on the composition of the systems studied. In this study, a complicated system such as crude RBO was studied, and SC-CO_2 extraction and fractionation process may be influenced by interactions among RBO components and individual solute solubilities in the supercritical phase.

Conclusions

CRUDE RBO CAN BE DEACIDIFIED UTILIZING SFF TECHNOLOGY without impairing the phytosterol content of the oil. Low-pressure and high-temperature processing were favorable for the crude-oil deacidification because under these conditions, phytosterol and TG losses with the extract fraction were lower, and FFA concentration of the extracts were higher. The phytosterol content, in particular oryzanol content, of the RBO deacidified using SC-CO_2 was about 3× higher than that found in a commercially available high-oryzanol RBO (Table 5). These results clearly indicate that SFF can be an alternative process for crude-oil deacidification and production of a phytosterol-enriched vegetable-oil extract.

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The authors wish to thank Mr. Leo Gingras of Riceland Foods Inc. for providing a generous supply of rice bran oil samples and technical information on conventional rice bran oil refining processes. We would also like to thank Mr. Jeff Teel for technical support, Dr. Fred Eller for his help on Statistix® software , and Dr. Robert Norton of the National Center for Agricultural Utilization Research, ARS/USDA, for providing oryzanol standards and personal communications on HPLC chromatograms of phytosterols.

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and use of the name by USDA implies no approval of the products and the exclusion of other that may also be suitable.

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